

# Anti-Cofilin/CFL1 Antibody Picoband™

Catalog Number: PB9033

#### **About CFL1**

Cofilin, a member of the ADF/cofilin family, is actually a protein with 70% sequence homology to ADF, making it part of the ADF/cofilin family of small ADP-binding proteins. It is a ubiquitous actin-binding factor required for the reorganization of actin filaments. The protein is known to sever actin filaments by creating more positive ends on filament fragments, it can cause depolymerization at the minus end of filaments, thereby preventing their reassembly. As a long-lasting in vivo effect, cofilin recycles older ADP-F-actin, helping cell to maintain ATP-G-actin pool for sustained motility. pH, phosphorylation and phosphoinositides regulate cofilin's binding and associating activity with actin.

#### Overview

Product Name	Anti-Cofilin/CFL1 Antibody Picoband™
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-Cofilin/CFL1 Antibody Picoband™ catalog # PB9033. Tested in ELISA, Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC, IHC-F, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P23528

#### **Technical Details**

Immunogen	E.coli-derived human Cofilin recombinant protein (Position: A2-L166). Human Cofilin shares 99% amino acid (aa) sequences identity with both mouse and rat Cofilin.
Predicted Reactive Species	Bovine, Horse, Monkey
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P), IHC(F) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized





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Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.  If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.  Some PubMed article(s) citing the expression level of this target are as follows:  Boster Bio's internal QC testing used:  Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat, Monkey  Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat, By Heat  Immunocytochemistry/Immunofluorescence, 2ug/ml, Human  Flow Cytometry, 1-3ug/1x10 <sup>6</sup> cells, Human  ELISA, 0.1-0.5ug/ml, Human, -



### Anti-Cofilin/CFL1 Antibody Picoband™ (PB9033) Images

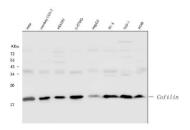


Figure 1. Western blot analysis of Cofilin using anti-Cofilin antibody (PB9033).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,

Lane 2: monkey COS-7 whole cell lysates,

Lane 3: human HEK293 whole cell lysates,

Lane 4: human U-87MG whole cell lysates,

Lane 5: human HepG2 whole cell lysates,

Lane 6: human PC-3 whole cell lysates,

Lane 7: human THP-1 whole cell lysates,

Lane 8: human A549 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-Cofilin antigen affinity purified polyclonal antibody (Catalog # PB9033) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for Cofilin at approximately 19 kDa. The expected band size for Cofilin is at 19 kDa.

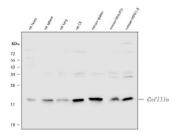


Figure 2. Western blot analysis of Cofilin using anti-Cofilin antibody (PB9033).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: rat heart tissue lysates,

Lane 2: rat spleen tissue lysates,

Lane 3: rat lung tissue lysates,

Lane 4: rat C6 whole cell lysates,

Lane 5: mouse spleen tissue lysates,

Lane 6: mouse NIH/3T3 whole cell lysates,

Lane 7: mouse HEPA1-6 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-Cofilin antigen affinity purified polyclonal antibody (Catalog # PB9033) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for Cofilin at approximately 19 kDa. The expected band size for Cofilin is at 19 kDa.



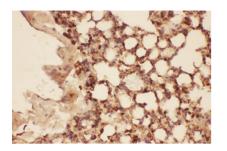


Figure 3. IHC analysis of Cofilin using anti-Cofilin antibody (PB9033).

Cofilin was detected in paraffin-embedded section of mouse lung tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Cofilin Antibody (PB9033) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

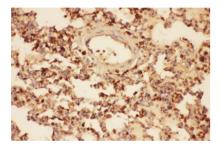


Figure 4. IHC analysis of Cofilin using anti-Cofilin antibody (PB9033).

Cofilin was detected in paraffin-embedded section of rat lung tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Cofilin Antibody (PB9033) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

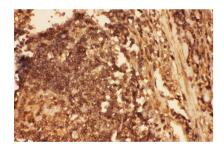


Figure 5. IHC analysis of Cofilin using anti-Cofilin antibody (PB9033).

Cofilin was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Cofilin Antibody (PB9033) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

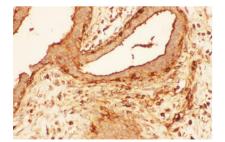
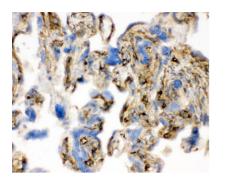


Figure 6. IHC analysis of Cofilin using anti-Cofilin antibody (PB9033).

Cofilin was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Cofilin Antibody (PB9033) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

Figure 7. IHC analysis of Cofilin using anti-Cofilin antibody (PB9033).





Cofilin was detected in frozen section of human placenta tissues. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Cofilin Antibody (PB9033) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

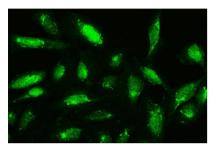


Figure 8. IF analysis of Cofilin using anti-Cofilin antibody (PB9033).

Cofilin was detected in immunocytochemical section of U20S cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-Cofilin Antibody (PB9033) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

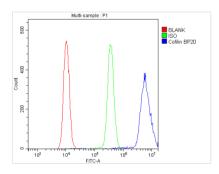


Figure 9. Flow Cytometry analysis of U20S cells using anti-Cofilin antibody (PB9033).

Overlay histogram showing U20S cells stained with PB9033 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Cofilin Antibody (PB9033,  $1ug/1x10^6$  cells) for 30 min at  $20^\circ$ C. DyLight® 488 conjugated goat anti-rabbit IgG (BA1127, 5- $10ug/1x10^6$  cells) was used as secondary antibody for 30 minutes at  $20^\circ$ C. Isotype control antibody (Green line) was rabbit IgG ( $1ug/1x10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

# **1 Publications Citing This Product**

1. PubMed ID: 30738249, Zhang J,Zhuang R,Zhang X,Hu W,Cheng K,Jiang D,Shen S,Zhang Y,Ding Y,Zhang Y.CD226 is involved in megakaryocyte activation and early-stage differentiation. Mol Immunol. 2019 Mar; 107:123-131. doi:10.1016/j.molimm.2019.01.013. Epub 2019 Feb 6. PMID: 30738249.

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